# ADENYLATE KINASE IN THE INTERCONVERSION OF ADENINE NUCLEOTIDES IN MUSCLE CYTOSOL

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The considerable significance of adenine nucleotides as substrates and regulators of metabolic processes in living cells is well documented [1]. In proportion to this importance, particularly that of AMP as metabolic regulator, information on subcellular distribution of adenylate kinase is rather insufficient and in some cases contradictory. Adelman et al. [2] reported recently the absence of adenylate kinase in the 100,000 *g* supernatants of intestinal mucosa, heart and skeletal muscle homogenates. We have found in the soluble fraction of rat heart muscle as much as 80% of the total adenylate kinase recovered, as shown in table 1. Glucosephosphate isomerase and glutamate dehydrogenase were also estimated, as markers of cytoplasmic and mitochondrial enzymes respectively. The fact that 40% of the glutamate dehydrogenase was found in the soluble fraction suggests considerable damage of mitochondria in the homogenization. Nevertheless, it seems clear that the subcellular distributions of glutamate dehydrogenase and adenylate kinase are markedly different. Though the proportion of adenylate kinase found in the soluble fraction is almost as great as that of glucosephosphate isomerase, the different behaviour of these enzymes after treatment with Triton X-100 suggests that, whereas the activity of glucosephosphate isomerase found in the particulate fraction is merely a contamination of cytosol, some adenylate kinase is bound to particulate structures in the homogenate. A similar subcellular distribution was also found for skeletal muscle in similar conditions, with total adenylate kinase activity more than twice that of heart muscle.

It is known that, after homogenization of a tissue,

Table 1
Subcellular distribution of adenylate kinase in heart and skeletal muscle.

| Enzymes | Subcellular fraction | | | |
|---|---|---|---|---|
| | Particulate | | Soluble | |
| | | Triton X-100 | | Triton X-100 |
| | (units/g muscle) | | | |
| *Heart* | | | | |
| Adenylate kinase | 7.7 | 11.5 | 53 | 54 |
| Glucosephosphate isomerase | 3.4 | 3.7 | 30 | 30 |
| Glutamate dehydrogenase | 1.6 | 3.1 | 2.4 | 2.2 |
| *Skeletal muscle* | | | | |
| Adenylate kinase | 37 | | 135 | |



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some enzymes are found bound to particulate structures of the cell or free as soluble enzymes, depending on the extraction conditions. This seems to be the case of adenylate kinase from liver, which Kielley and Kielley [3] reported to be located almost exclusively in mitochondria, while Heldt and Schwalbach [4] found 22% of the total activity in the supernatant fraction and an additional 60% that could be extracted by treatment of the particulate material with 0.1 M phosphate. Adelman et al. [2] found virtually all the liver adenylate kinase activity in the supernatant without treatment with phosphate and ascribed the dissimilarity between their results and those of Heldt and Schwalbach [4] to slight differences in the methodology used. Presumably the absence of adenylate kinase from the supernatant fractions of heart and skeletal muscle reported by Adelman et al. [2] could also have been a consequence of some particular condition. In our experience, carrying out the homogenization of either heart or skeletal muscle with 0.25 M sucrose instead of the saline solution resulted also in the large majority of the adenylate kinase being found in the soluble fraction.

Sottocasa et al. [5] have reported that the adenylate kinase in mitochondria from liver is located in the space between the inner and the outer mitochondrial membranes or weakly bound to the outer membrane. The same location has been found by Vallejo et al. [6] in the case of the enzyme from brain, in which only 5% was found to be associated with the mitochondrial fraction. Our results suggest that no marked differences in the subcellular location of adenylate kinase exist between skeletal or heart muscle and liver or brain. On the basis of present knowledge, three possibilities could account for these results: a) adenylate kinase is weakly bound to the outer mitochondrial membrane and released in various degrees depending on the extraction procedure; b) adenylate kinase is free in the cytosol but binds to the outer mitochondrial membrane in the homogenate; c) there is an equilibrium in the cell between adenylate kinase free in the cytosol and adenylate kinase bound to the outer membrane. A predominant location in the cytosol is the more likely possibility. In any case, interconversion of adenine nucleotides in the cytosol would be ensured since in any of the above possibilities the substrates could easily reach the enzyme.

The identification of a high activity of adenylate kinase in muscle cytosol can account for the maintenance of the three adenine nucleotides near equilibrium, thus making possible the rapid involvement of AMP-mediated allosteric activations of key enzymes of glycogenolysis [7] and glycolysis [8] that are very important at the onset of intense muscular activity.

White rats were killed by decapitation. Heart muscle was dissected, passed three times through a meat grinder with holes of 1 mm diameter, and homogenized by hand with a Teflon pestle, with 5 volumes of a saline solution containing 5 mM phosphate, 1 mM dithioerythritol, 150 mM KCl, 5 mM $KHCO_3$, 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$, adjusted to pH 7.0. The homogenate was centrifuged at 100,000 *g* for 30 min; after decantation of the soluble fraction, the pellet was resuspended in 10 volumes of the saline solution and filtered through gauze to remove cell debris, this filtrate being taken as the particulate fraction. All operations were carried out at 2–4°. Aliquots of each fraction were incubated 5 min with 1% Triton X-100 before assay of enzyme activities, when indicated. Adenylate kinase activity was assayed at room temperature (ca. 22°) following the formation of ATP (in a final volume of 1 ml) in 100 mM imidazole (pH 7.0), with 5 mM ADP (pH 7.0), 10 mM $MgCl_2$, 100 mM KCl, 1 mM glucose, 0.5 units of hexokinase, 0.5 units of glucosephosphate dehydrogenase, and 0.5 mM $TPN^+$; after waiting to remove the ATP contamination present in the ADP preparation, the reaction was started by addition of the 5 to 20 $\mu$l of the above fractions. Glucosephosphate isomerase activity was assayed following glucose-6-P formation, in 50 mM imidazole (pH 7.0), with 2 mM fructose-6-P, 0.5 mM $TPN^+$, and 0.5 units of glucosephosphate dehydrogenase. Glutamate dehydrogenase was measured following the formation of glutamate in 50 mM triethylamine (pH 8.0), with 10 mM $\alpha$-ketoglutarate, 100 mM $NH_4Cl$, 5 mM EDTA, 0.15 $\mu$M DPNH, 1.5 mM ADP and 1 mM KCN. Activities are expressed in $\mu$moles of substrate transformed per minute (ATP formed × 2 in the case of adenylate kinase). Skeletal muscle was minced with scissors and homogenized in a high speed Virtis homogenizer and fractionated as indicated above.

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